2071-Pos Board B57
Solid-State NMR Study on the Conductance Mechanism and Acid Activation of M2 Proton Channel
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M2 is a 97-residue, single-pass membrane protein which functions in a tetrameric state. The tetramer forms a proton channel which has been proven to be a drug target for *Influenza* A virus (H3N2 and H1N1). However, the M2 protein of this virus has recently mutated so that the anti-flu drugs, amantadine and rimantadine are no longer effective; consequently there is increased urgency to define the structure and functional mechanism of this protein as an aid for drug development. Even though it has long been believed that M2 protein is a proton channel with a high selectivity, most functional assays find a low conductance rate which resembles the activity of a transporter. An atomistic mechanism has recently been proposed for the proton transport involving the His37 and Trp41 residues in this tetramer [1]. Here we present magic angle spinning solid state NMR spectroscopic data of the M2(22-62) construct and the full-length protein that bear on this mechanistic model and on the pH-induced structural changes. Solid state NMR provides an opportunity to characterize the protein in a native-like lipid bilayer environment. Recently, a comparison of M2 transmembrane domain structures obtained from detergent and lipid bilayer environments has demonstrated the importance of using good models of the membrane environment [2].
[1] M. Sharma, M. Yi, H. Dong, H. Qin, E. Peterson, D. D. Busath, H.-X. Zhou, and T. A. Cross (2010). Insight into the mechanism of the influenza A proton channel from a structure in a lipid bilayer. Science (in press).
[2] T. A. Cross, M. Sharma, M. Yi, and H.-X. Zhou (2010). Influence of solubilizing environments on membrane protein structures. Trends Biochem. Sci. (in press).

2072-Pos Board B58
Structure and Topology of Phospholamban Bound to Ca^{2+}-ATPase in Lipid Bilayers Studied by Hybrid NMR Methods
Martin Gustavsson, Nathaniel J. Traaseth, Raffaello Verardi, Gianluigi Veglia.
Phospholamban (PLN) is an integral membrane protein that regulates Sarcoplasmic Reticulum Ca2+-ATPase (SERCA) in cardiomyocytes. Its structure and topology have been solved by a hybrid method of solution and solid-state NMR in detergent micelles and lipid bilayers. The combination of NMR restraints and explicit MD simulations in DOPC clearly reveals PLN's architecture: 1) a regulatory cytoplasmic helix (domain Ia) that is absorbed on the bilayer surface, 2) a hydrophillic helix (domain Ib) that hydrogen bonds with lipid head groups, and 3) a transmembrane helix (domain II) that spans the most hydrophobic part of the membrane. To build on the hybrid method and fully elucidate the structure and topology of PLN when bound to SERCA, we performed magic angle spinning (MAS) experiments combined with oriented solid-state NMR in lipid bilayers under fully functional conditions. This method eliminates the need for solution NMR in detergent micelles, which can distort the structures of membrane proteins. Since high degeneracy of hydrophobic amino acids in PLN (Leu, Val, Ile, Ala) leads to severe spectral overlap for uniformly 13C,15N-labeled samples, we used selective labeling to enable complete backbone and side chain assignments. The chemical shifts measured by MAS experiments show that the secondary structure of PLN is unchanged upon binding SERCA (i.e., domain II is still helical). However, we observed methyl group chemical shift perturbations (e.g., Ile 38-Cg2,Cd1) that are consistent with the SERCA-interacting face of PLN that was previously proposed from cross-linking data. Oriented NMR experiments showed that the domain II tilt angle of SERCA-bound PLN is conserved between magnetically aligned DMPC/D6PC bicelles and mechanically aligned DOPC/DOPE bilayers. The use of our hybrid approach to study the PLN/SERCA complex (~110 kDa) demonstrates its applicability to other membrane protein complexes.

2073-Pos Board B59
Tuning the Structural Coupling Between the Transmembrane and Cytoplasmic Domains of Phospholamban to Control SERCA Inhibition
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Phospholamban (PLN) is the endogenous inhibitor of the sarco(endo)plasmic reticulum Ca2+ ATPase (SERCA), the integral membrane enzyme responsible for 70% of the Ca2+ shuttling into the SR, inducing cardiac muscle relaxation in humans. Dysfunctions in SERCA:PLN interactions have been implicated as having a critical role in cardiac disease. Based on the success of the Chien Group with S16E PLN, a pseudo-phosphorylated PLN species which halted the progression of heart failure in animal models, we wish to further develop PLN species to improve SERCA function by rationally designing mutations based on the structural and biophysical data available on the system. The present study seeks to introduce single and multiple glycine mutations in the loop domain of PLN in order to tune the structural coupling between the cytoplasmic and transmembrane helix, and ascertain the effects on SERCA function. These studies further develop the model by which the control of enzyme function is performed by altering the structural dynamics of a small inhibitor can then be translated to other membrane enzymes, such as the Na/K-ATPase.

2074-Pos Board B60
Mycobacterium Tuberculosis Protein Rv1861: Structural Insights into GTP Hydrolysis in a Native-Like Membrane Environment
Dylan T. Murray, Nabanita Das, Timothy A. Cross.
1/3 of the world's population is infected with Mycobacterium tuberculosis 10% of whom will become sick from the bacilli. Multidrug resistant strains resistant to the leading tuberculosis antibiotics, isoniazid and rifampicin have emerged necessitating new treatments. Integral membrane proteins are an excellent source of novel drug targets. Structural biology can provide rich information regarding the influence of lead compounds on protein function. Solid state nuclear magnetic resonance is increasingly being used to understand membrane protein structure and dynamics in lipid membrane environments. The structural information can be accompanied with other biophysical techniques to make conclusions regarding membrane protein function and the influence of regulatory compounds. Rv1861 is an integral membrane protein from Mycobacterium tuberculosis. It contains the signature GTP binding domain motif AXXXXGKT near the N-terminus of the protein and is predicted to contain three transmembrane alpha helices from hydrophobicity analysis.
Oriented sample solid state magnetic resonance accurately measures peptide plane orientations through PISEMA experiments on protein in liquid crystalline lipid bilayers aligned between glass slides. The structural data can be correlated to functional information from experiments such as isothermal titration calorimetry on the protein in liposomes allowing protein structure and function to be understood in a native-like environment. Here we present the initial structural characterization of the Rv1861 protein in lipid bilayers. PISEMA experiments on uniformly and amino acid type specifically labeled samples are used to determine helix orientation. Light scattering, size exclusion chromotography and electrophoresis experiments on the protein in micellar environments will provide the oligomeric state and compliment the secondary structure characterization. The data provide a firm foundation for further structure determination and functional characterization of the protein in a native-like environment.

2075-Pos Board B61
Understanding the Potential Interaction of Transmembrane Helices from MgtC and MgtR
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Beyond the challenge consisting of solving a membrane protein structure, Solid state NMR is the most powerful technique to decipher protein dynamics within the membrane hydrophobic core. Recent results have converged to highlight the role of hydrophobic peptides that form a novel class of active molecules in *Escherichia coli* and *Salmonella enteric serovar Typhimurium*. These peptides are apparently able to interact with membrane proteins leading to their inactivation through an unfolding process. Understanding the molecular mechanism of these peptide-protein interactions would offer great opportunities in pharmacology and drug design.
MgtR, a highly hydrophobic 30 amino acids peptide, which is expressed in *Salmonella typhimurium,* is one of these active molecules. It has been shown that within the macrophage, the over expression of MgtR leads to a decrease of Salmonella replication rate. This process was shown to involve an interaction between MgtR and a membrane protein of *Salmollena* (MgtC) which is required for the bacteria survival at low magnesium concentration. Throughout the literature, MgtC has proven to be one of the key factors in *Mycobaterium tuberculosis* latent state. Relying on the sequence homology between *Salmonella typhimurium* MgtC and *Mycobacterium tuberculosis* MgtC, the interaction between MgtR from *Salmonella typhimurium* and the transmembrane domain #4 of *Mycobacterium tuberculosis* MgtC will be studied.
Structural studies using solid state NMR methods such as CP-MAS and PI SEMA on oriented samples have been performed. Several ^{15}N labeled peptides were produced by chemical synthesis and biological expression prior reconstitution into model membranes. These NMR studies allowed us to determine theses peptides topology and their backbone structure within membranes.